ON THE INFLUENCE OF METEOROLOGICAL CONDITIONS ON THE DEVELOPMENT OF *TRYPANOSOMA RHODESIENSE* IN *GLOSSINA MORSITANS**

BY

ALLAN KINGHORN

AND

WARRINGTON YORKE

(*Fifth interim report of the Luangwa Sleeping Sickness Commission of the British South Africa Company*)

(*Received for publication* 23 *September*, 1912)

In a former report†, attention was drawn to certain points which seemed to indicate that meteorological conditions, in the Luangwa Valley, had a considerable influence on the development of *Trypanosoma rhodesiense* in *Glossina morsitans*. As, however, the experiments were few in number, and were carried out during only a single dry and wet season, no definite conclusions could be drawn. Since the Commission established its headquarters on the Congo-Zambesi watershed, evidence has accumulated which indicates decisively that such conditions, more particularly the temperature, exert a very pronounced influence on the development of the trypanosome in *Glossina morsitans*.

In Tables 1 and 2 the meteorological conditions at Nwalia (Luangwa Valley) and at Ngoa (Congo-Zambesi Watershed) are summarised.

At Nawalia, eight transmission experiments were made, four with laboratory-bred, and four with 'wild' *Glossina morsitans*. At Ngoa, five experiments have been carried out to date, four with 'wild,' and one with bred flies. It is unfortunate that the bred

* Reprinted with additions from the Brit. Med. Journ., October 5th, 1912.

† Kinghorn and Yorke, Annals Trop. Med. and Parasit., Vol. VI, p. 275, July, 1912.

TABLE 1.—Meteorological observations at Nawalia, N. Rhodesia, 12° 25′ S., 32° 2′ E., altitude 2,100 feet (approximately).

1911-1912	External shade tem. mean	Absolute maximum	Absolute minimum	Laboratory tem. mean	Relative humidity per cent.	Rainfall inches	Days on which rain fell
June	67·2	89·0	44·3	—	48·6	0	—
July	68·7	91·0	44·6	67·4	45·7	0	—
August	73·3	96·8	51·2	71·2	35·8	0	—
September ...	77·5	103·3	54·0	71·5	31·5	0	—
October.....	86·1	107·6	62·3	84·5	31·8	0·26	2
November.....	87·1	107·8	67·9	84·6	41·1	1·61	8
December ...	82·3	101·7	67·8	79·6	69·1	8·54	20
January	80·6	101·1	67·0	78·4	77·7	14·97	16
February	79·2	96·0	66·1	77·1	73·8	5·55	16
March	79·0	97·6	61·0	72·0	62·5	5·10	6
April (to 9th)	79·5	97·9	59·8	77·2	58·0	0·01	1
						36·04	69

TABLE 2.—Meteorological observations at Ngoa, N. Rhodesia, 11° 40′ S., 31° 30′ E., altitude 4,400 feet (approximately).

1912	External shade tem. mean	Absolute maximum	Absolute minimum	Laboratory tem. mean	Relative humidity per cent.	Rain fall inches	Days on which rain fell
April (20th—30th)	72·0	89·7	54·1	68·0	53·7	0	—
May	69·0	90·5	45·1	66·2	51·3	0	—
June	61·6	85·5	34·3	60·5	53·0	0·01	1
July	62·4	83·0	42·3	60·9	53·0	0	—
						0·01	1

flies are not more numerous, but owing to the low temperature the majority of the flies did not emerge from the puparia and many of those which did were malformed, and quickly died. In all the experiments in which 'wild' flies were used, however, the possibility that they were already infected with the trypanosome was excluded by first feeding them on healthy monkeys.

The details of the Nawalia experiments have already been published*, while those of the Ngoa series will appear in a later report. For the purposes of the present paper synopses of the two series will suffice, and will be found in Tables 3 and 4.

TABLE 3.—Synopsis of transmission experiments with *Trypanosoma rhodesiense* and *Glossina morsitans* carried out at Nawalia, N. Rhodesia.

No.	Date on which started	Season	Number flies used	Variety flies used	Result	Duration of developmental cycle in days	Mean temperature during developmental cycle†	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity† per cent.
1	20/8/11	Dry	26	bred	Infection	13	75·1	90·0	60·5	35·0
2	14/11/11	Commencement of rains	16	,,	,,	15	83·5	93·5	74·5	44·8
3	14/11/11	,,	57	wild	,,	11	83·8	93·5	74·5	44·8
4	29/12/11	Rainy	20	bred	Negative	—	78·2	87·1	72·8	74·5
5	12/1/12	,,	42	wild	,,	—	78·0	85·7	72·8	74·2
6	12/1/12	,,	42	,,	,,	—	78·0	85·7	72·8	74·2
7	12/1/12	,,	23	bred	Infection	19	77·9	85·7	72·8	75·4
8	13/2/12	,,	104	wild	,,	25	77·3	86·0	71·0	66·5

It will be seen from these tables that, whereas in the Luangwa Valley, *Trypanosoma rhodesiense* was successfully transmitted by *Glossina morsitans*, all efforts in this direction on the Congo-Zambesi watershed have so far been in vain. Of 330 flies used in the Valley experiments, six, and probably ten, became infective. The larger figure is based on the number of salivary gland infections found in the flies. Our experience indicates that the

* Kinghorn and Yorke, Annals Trop. Med. and Parasit., Vol. VI, No. 1 A, 1912.

† In the case of the unsuccessful experiments, the mean temperature, and the relative humidity have been calculated for the first 30 days only.

implication of these structures is intimately connected with the ability of *Glossina morsitans* to transmit *Trypanosoma rhodesiense*, and that until they are invaded by the organisms the flies are non-infective. Salivary gland infections have been found in all the flies which were capable of transmitting the parasite. In Experiment 3 (Table 3) six flies were found to harbour trypanosomes in the glands, but of these only two were actually proved to transmit *Trypanosoma rhodesiense*. As in all other instances it was shown conclusively that those flies in which trypanosomes were found in the salivary glands were infective, it may be concluded that the remaining four flies in this experiment were also capable of transmitting the parasite. Invasion of the salivary glands has not been observed, except in those flies which were known to transmit *Trypanosoma rhodesiense*.

TABLE 4.—Synopsis of transmission experiments with *Trypanosoma rhodesiense* and *Glossina morsitans*, carried out at Ngoa, N. Rhodesia.

No.	Date on which started	Season	Number flies used	Variety flies used	Result	Duration of developmental cycle in days	Mean temperature during developmental cycle	Absolute maximum during development cycle	Absolute minimum during developmental cycle	Relative humidity per cent.
1	18/5/12	Dry	116	Wild	Negative	—	62·7	74·5	50·5	52·0
2	13/6/12	,,	90	,,	,,	—	59·1	72·4	42·0	52·0
3	14/6/12	,,	119	,,	,,	—	59·1	72·4	42·0	52·0
4	26/6/12	,,	19	Bred	,,	—	60·2	71·8	42·0	52·0
5	11/7/12	,,	176	Wild	,,	—	62·0	72·0	49·1	50·0

In the five plateau experiments, 520 *Glossina morsitans* were employed without a single fly becoming infective.

The explanation of these apparently contradictory results is at first sight not very obvious, more particularly in view of the fact that even on the plateau, 'wild' *Glossina morsitans* capable of infecting healthy monkeys with *Trypanosoma rhodesiense* were occasionally encountered. If the climatic conditions under which the valley experiments were carried out be compared with those

obtaining during the plateau experiments, it will be seen at once that the most striking difference is one of temperature. As a rule, the temperature during the former series of experiments was roughly from 15-20° F. higher than during the latter series.

With a view to ascertaining the influence, if any, exerted by temperature on the developmental cycle of *Trypanosoma rhodesiense* in the tsetse fly, a further series of experiments were performed on the plateau, in which, by means of an incubator, the flies were kept at a temperature approximating to that of the valley at the most favourable season.

Table 5.—Synopsis of experiments to transmit *Trypanosoma rhodesiense* by means of *Glossina morsitans* kept in incubator.

No.	Date on which started	Season	Number flies used	Variety flies used	Result	Duration of developmental cycle in days	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity per cent.
1	30/6/12	Dry	61	Wild	Infection	14	80·6	87·8	74·5	36·0*
2	1/7/12	,,	72	,,	,,	13	80·6	87·8	74·5	36·0*

* Approximate.

No water was placed in the incubator, and the warm, dry air was found to have a very deleterious effect on the insects. Within the first seven days, 25 of the 61 flies with which Experiment No. 1 was commenced, and 53 of the 72 in Experiment No. 2 had died. Notwithstanding the small number alive at the end of the second week, two infective flies were obtained in Experiment 1 and one in Experiment 2.

It seems obvious, therefore, that a high temperature favours the development of the trypanosome in *Glossina morsitans*, and that the comparatively low temperatures obtaining during May, June and July on the Congo-Zambesi watershed are very unfavourable to the completion of the developmental cycle of *Trypanosoma rhodesiense* in this fly, and would account for the unsuccessful experiments at laboratory temperatures.

Other observations afford corroborative evidence in support of this contention. An analysis of the valley transmission experiments shows that the largest number of infective flies was obtained at the hottest season of the year.

TABLE 6—Showing number of infective flies obtained, and meteorological observations at Nawalia (Luangwa Valley)

No.	Date on which started	Season	Duration of developmental cycle in days	Number infective flies found	Mean temperature during developmental cycle	Absolute maximum during developmental cycle	Absolute minimum during developmental cycle	Relative humidity per cent.
1	20/8/11	Dry	13	1	75·1	90·0	60·5	35·0
2	14/11/11	Commencement of rains	15	1	83·5	93·5	74·5	44·8
3	14/11/11	,,	11	6	83·8	93·5	74·5	44·8
4	29/12/11	Rainy	—	0	78·2	87·1	72·8	74·5
5	12/1/12	,,	—	0	78·0	85·7	72·8	74·2
6	12/1/12	,,	—	0	78·0	85·7	72·8	74·2
7	12/1/12	,,	19	1	77·9	85·7	72·8	75·4
8	13/2/12	,,	25	1	77·3	86·0	71·0	66·5

The fact that an occasional infective 'wild' fly was encountered on the plateau during a period (May, June and July) when attempts to transmit in the laboratory were invariably unsuccessful requires some explanation. A possible solution may be that the flies in question were infected during the warmer season of the year and had survived into the cold season.

If the results obtained by feeding freshly-caught flies on healthy monkeys in the valley are compared with those from flies caught on the plateau, a marked difference in the number of infections resulting is apparent. In the Luangwa Valley, 3,202 flies were fed in 29 batches and *Trypanosoma rhodesiense* was isolated in six of the experiments, giving a ratio of 1 infective fly to 534, whereas on the Congo-Zambesi watershed, 5,041 freshly-caught *Glossina morsitans* were fed in groups on 39 monkeys, with four positive results—1 infective fly to 1,260. As tsetse flies and game

are about equally numerous at Nawalia and Ngoa, and as the disease was presumably introduced into the two localities, which are less than 70 miles apart, about the same time, it appears to us that the only essential difference which can account for the fact that the percentage of infective 'wild' flies at Nawalia is two and a half times as great as at Ngoa is the difference in the climatic conditions. It will be seen from Tables 1 and 2 that the temperatures experienced on the Congo-Zambesi watershed during May, June and July are very much lower than those at Nawalia from September to March. It was during the months named that our experiments were carried out at the two places.

Finally, it might be mentioned that the percentage of infective 'wild' flies caught in the valley was greater in the hot than in the cold season. This point is illustrated in Table 7.

TABLE 7—Percentage of *Glossina morsitans* found infected with *Trypanosoma rhodesiense* at Nawalia at different seasons of the year.

1911-1912	Mean external shade temperature	Number flies fed	Number infections with *T. rhodesiense*	Ratio of infective to non-infective flies
June...	67·2	18	0	
July	68·7	385	0	
August	73·3	193	0	0 : 790
September	77·5	194	0	
October	86·1	—	—	
November	87·1	270	1	
December	82·3	205	0	1 : 338
January	80·6	538	2	
February	79·2	104	0	
March	79·0	823	2	1 : 466
April (to 9th) ...	79·5	472	1	

In addition to temperature, there is one other factor in the climatic conditions which may possibly influence the developmental cycle of the trypanosome in *Glossina morsitans*. We refer to the relative humidity of the atmosphere. At the most favourable

season of the year in the Luangwa Valley for transmission experiments, and also in the case of those carried out in the incubator, the relative humidity was extremely low. Whether this is a mere coincidence we are, at present, unable to form an opinion, but hope to be able to write more definitely on the subject in a later communication.

SUMMARY

The developmental cycle of *Trypanosoma rhodesiense* in *Glossina morsitans* is, to a marked degree, influenced by the temperature to which the flies are subjected. High temperatures (75-85° F.) favour the development of the parasite, whilst low temperatures (60-70° F.) are unfavourable.

NGOA, N. RHODESIA,
August 3, 1912.

ADDENDUM

Since writing the above, a further experiment has been completed which, besides affording support to the conclusions arrived at, indicates in a rather more definite manner the influence of temperature on the developmental cycle of *Trypanosoma rhodesiense* in *Glossina morsitans*.

Two batches of 'wild' *Glossina morsitans* (Batch A consisting of 90 flies and Batch B of 119) in which the possibility of the presence of an infective fly had been previously excluded by feeding the insects on clean monkeys, were fed for three consecutive days on a guinea-pig infected with *Trypanosoma rhodesiense*. After being starved for a day, each batch was fed on a healthy monkey until the 40th day after the first feed on the infected animal. Neither of the monkeys became infected. Batch A, in which there were then 42 flies still alive, was placed in the incubator, whilst Batch B, in which there were now 58 flies, was kept as before at laboratory temperature. The sudden change from the laboratory to the warm, dry air of the incubator proved very fatal to the flies in Batch A, and on the 43rd day only six were alive. From the 41st to the 47th day the flies in this batch were fed on a monkey,

and from the 48th day on a rat. The rat became infected on the 53rd day, so that allowing five days for the incubation of the disease in the animal, Batch A contained an infective fly on the 48th day after the first feed on the infected guinea-pig, and eight days after being placed in the incubator. As the monkey died on the 47th day, we are unable to state whether the fly became infective before the 48th day. The four flies still living on the 53rd day were fed on four clean rats, and three of these became infected.

The monkey on which Batch B was fed was still negative at the end of 60 days, when there were 38 flies alive.

From this experiment it appears that the first part of the developmental cycle of *Trypanosoma rhodesiense* in *Glossina morsitans* can occur at comparatively low temperatures (60° F.), whereas a considerably higher temperature (75-85° F.) is necessary for the completion of the cycle.

It is interesting to note that the flies in Batch A became infective eight days (possibly less) after being placed in the incubator. This is three days less than the shortest incubation period observed in any of our successful transmission experiments,—a fact which supports the view that the developmental cycle of the parasite in the fly had proceeded to a certain point at the laboratory temperature (60° F.) before the insect was subjected to the higher temperature (80° F.) of the incubator.

NGOA, N. RHODESIA,
August 10, 1912.